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Comparison of Four Commercial Brucella Agar Media for Growth of Anaerobic Organisms

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Four different commercial brucella blood agar plating media (Anaerobe Systems, BBL Microbiology Systems, Remel, and Scott Laboratories) were compared for the abilities to recover anaerobic organisms from clinical specimens and to support the growth of American Type Culture Collection anaerobic stock cultures. Following 24 h of incubation in an anaerobe chamber, Anaerobe Systems prereduced, anaerobically sterilized brucella plates yielded 63% of the total clinical anaerobe isolates, the Scott medium yielded 51%, the Remel medium yielded 42%, and the BBL medium yielded 37%. Poor growth of *Peptostreptococcus magnus*, *P. anaerobius*, *Fusobacterium necrophorum*, *F. nucleatum*, and pigmented *Bacteroides* spp. was observed on brucella media obtained from BBL, Remel, and Scott. Data obtained with stock anaerobic cultures showed that Anaerobe Systems plates yielded good growth and produced a larger colony size with all of the strains tested in 1 day, whereas poor growth of *Peptostreptococcus* spp., *B. melaninogenicus*, and *Fusobacterium* spp. was noted on brucella media from BBL, Remel, and Scott.

Clinical laboratories must have the capability to recover anaerobic bacteria rapidly from specimens to assist physicians with the proper diagnosis and treatment of patients with anaerobic infections. Recovery of anaerobes from clinical specimens depends on (i) the procedure of specimen collection and transportation, (ii) the anaerobic incubation system used, and (iii) the quality and selection of the primary isolation media (3, 10, 11). Currently, there are a number of improvements in the collection, transport, and incubation of clinical specimens of anaerobes (3, 7, 8, 11, 15). However, there are conflicting guidelines as to the proper selection of an optimum plating medium for the recovery and growth of anaerobes (6, 9, 10, 13, 14). Some studies suggest that freshly prepared, properly stored, highly enriched media are essential for recovery of anaerobes (6, 8, 14), while other studies have shown that prereduced, anaerobically sterilized (PRAS) media best support the growth of anaerobes (7, 9, 12). Prereduced and PRAS are terms for media that are sterilized in a reduced condition and remain reduced up to and including the time when they are inoculated (7). The preparation of these media is impractical for most clinical laboratories; therefore, commercially prepared anaerobic media are used, although the abilities of such media to support the growth of anaerobes have not been extensively studied.

The purpose of this study was to determine whether plating media that are compounded, autoclaved, poured, packaged, and stored under anaerobic conditions, i.e., PRAS media (Anaerobe Systems, Santa Clara, Calif.), are less toxic and therefore more efficient than media not manufactured under these stringent guidelines (BBL Microbiology Systems, Cockeysville, Md.; Remel, Lenexa, Kans.; and Scott Laboratories, Inc., Fiskeville, R.I.). Each of the four brucella plates was compared (i) to determine the ability of each medium to grow anaerobic isolates from clinical specimens and (ii) to determine the presence of growth, colony size, and rate of growth of 12 American Type Culture Collection (ATCC; Rockville, Md.) anaerobic stock cultures on each medium.

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MATERIALS AND METHODS

Clinical specimens. Clinical specimens were obtained from patients at Good Samaritan Hospital, San Jose, Calif. The specimens submitted were either tissue placed in an anaerobic transport vial (Port-A-Cul; BBL) abscess aspirate (obtained with a needle and syringe) or drainage collected by swab and placed in an anaerobic transport tube (Port-A-Cul). Each specimen was collected and transported immediately to the clinical laboratory. Most of the specimens obtained were from patients with abscesses (intra-abdominal, liver, peritoneal, lung, and brain), empyema, soft tissue infections, cellulitis, and female genital tract infections. Contamination with normal flora was avoided.

Processing. Upon receipt in the clinical laboratory, specimens were placed immediately inside an anaerobic chamber (Anaerobe Systems), plated on the four different brucella blood agar plates, and placed in an incubator within the anaerobic chamber. Incubation of all plates was at 35°C (the atmosphere of the chamber and incubator was 85% N_2–10% CO_2–5% H_2). A sample from each specimen was inoculated onto each plate as follows. A 0.1-ml portion of each fluid specimen was pipetted onto each test medium; tissue samples were ground in 0.5 ml of PRAS brain heart infusion broth (Anaerobe Systems), and 0.1 ml was inoculated onto each plate; swabs were vortexed inside the anaerobic chamber in 0.5 ml of PRAS brain heart infusion broth, and 0.1 ml was inoculated onto each plate. Plates were uniformly streaked to allow a semiquantitative estimate (1+, 2+, 3+, or 4+; 1+ is rare growth, and 4+ is heavy growth) of the number of organisms present. Plates were evaluated daily for 3 days. If a plating medium was overgrown with swarming *Proteus* sp. or *Clostridium* sp., that specimen was not included in the study.

Plating media. Four commercially prepared brucella blood agar plates were selected because their compositions are identical (brucella agar base, 5% defibrinated sheep blood, hemin, and vitamin K). The media were purchased from Anaerobe Systems, BBL, Remel, and Scott Laboratories. Anaerobe Systems manufactures and packages their medium anaerobically (PRAS), while the BBL, Remel, and Scott media are manufactured and packaged aerobically in cello-

TABLE 1. Recovery of clinical anaerobic isolates on four different brucella media

Organism	Total no. of isolates recovered	% Recovered by medium from the following manufacturer (days 1, 2, and 3):			
		Anaerobe Systems	BBL	Remel	Scott
Bacteroides fragilis	18	72, 94, 100	44, 50, 78	50, 50, 78	61, 67, 83
B. thetaiotaomicron	16	56, 100, 100	37, 44, 69	37, 56, 75	50, 75, 87
B. vulgatus	12	67, 75, 75	42, 50, 67	50, 58, 75	83, 83, 100
B. distasonis	4	50, 100, 100	50, 50, 50	50, 50, 50	50, 50, 50
B. melaninogenicus group	8	25, 62, 100	0, 25, 37	0, 0, 25	0, 12, 25
B. disiens	4	75, 100, 100	25, 25, 50	25, 25, 75	50, 50, 50
B. bivius	6	83, 83, 100	33, 33, 66	17, 17, 66	50, 50, 100
Fusobacterium nucleatum	5	60, 100, 100	0, 0, 60	0, 0, 40	20, 20, 60
F. necrophorum	5	40, 60, 100	0, 0, 20	0, 0, 40	0, 0, 40
F. mortiferum	4	50, 50, 100	0, 0, 25	0, 0, 25	0, 25, 50
Clostridium perfringens	5	80, 80, 80	80, 80, 80	80, 80, 100	80, 80, 100
C. ramosum	3	66, 66, 100	33, 66, 66	33, 66, 66	66, 66, 66
C. innocuum	2	100, 100, 100	100, 100, 100	50, 100, 100	100, 100, 100
C. difficile	3	66, 66, 66	66, 66, 66	66, 66, 100	100, 100, 100
Other clostridia	5	80, 80, 100	80, 80, 80	80, 80, 80	80, 80, 100
Peptostreptococcus anaerobius	4	50, 100, 100	0, 0, 50	25, 25, 25	0, 25, 50
P. asaccharolyticus	4	50, 75, 100	25, 25, 25	25, 25, 25	25, 25, 50
P. magnus	2	50, 100, 100	0, 0, 0	0, 0, 0	50, 50, 50
Veillonella parvula	2	50, 50, 50	0, 50, 50	0, 50, 100	50, 50, 100
Eubacterium limosum	2	100, 100, 100	0, 0, 50	0, 0, 0	0, 50, 50
Propionibacterium acnes	3	66, 100, 100	0, 33, 66	33, 66, 66	66, 100, 100
Total no. or % recovered	117	61, 85, 94	32, 38, 56	34, 41, 61	49, 57, 75

phane wrappers. All of the media were used within 2 weeks of receipt and at least 4 to 6 weeks before expiration. Manufacturer instructions were followed for reduction of the media. Scott, Remel, and BBL plates were stored in a refrigerator (2 to 8°C), and when needed they were brought to room temperature, placed into the anaerobic chamber, and reduced for a minimum of 24 h before inoculation. Anaerobe Systems PRAS plates were stored at room temperature in their aluminum foil pouches as recommended by the manufacturer and placed into the anaerobic chamber when needed. Prior reduction of Anaerobe Systems PRAS plates was not necessary.

Identification of anaerobic isolates. Each colony type from each brucella plate was (i) picked for Gram staining, (ii) subcultured to the appropriate commercial brucella plate for isolation and further testing, and (iii) subcultured to a chocolate agar plate (Remel) for aerotolerance testing.

Aerotolerance testing. For aerotolerance testing, chocolate agar plates were incubated for 48 h in a CO_2 incubator and evaluated for growth.

Presumptive identification. Presumptive identification was done by using special-potency antibiotic disks (Difco Laboratories, Detroit, Mich.) and other rapid identification methods as outlined in reference 15.

Final identification. Final identification was done by using the RapID-ANA (Innovative Diagnostics, Atlanta, Ga.) 4-h identification system.

ATCC organisms. The following 12 ATCC anaerobic stock cultures, which varied in their nutritional and anaerobic requirements, were used: *Bacteroides fragilis* ATCC 25285, *B. melaninogenicus* ATCC 15930, *B. ovatus* ATCC 8483, *B. thetaiotaomicron* ATCC 29741, *Clostridium difficile* ATCC 9689, *Fusobacterium mortiferum* ATCC 25557, *F. necrophorum* ATCC 25286, *F. nucleatum* ATCC 25586, *Peptostreptococcus anaerobius* ATCC 27337, *P. asaccharolyticus* ATCC 14963, *P. magnus* ATCC 29328, and *Propionibacterium acnes* ATCC 1827. The ATCC cultures were maintained at −70°C in skim milk. When needed, they were passed into the anaerobe chamber, allowed to thaw, subcultured to brucella medium (Anaerobe Systems), and incubated overnight in the chamber incubator. A PRAS-brucella broth (Anaerobe Systems) suspension was prepared and adjusted to match a 0.5 McFarland standard. A standard 0.001-ml calibrated loopful of the suspension was used to streak the plates to obtain semiquantitative growth and isolated colonies. All procedures were conducted in an anaerobe chamber, and all plates were placed in an incubator contained in the anaerobe chamber set at 35°C. The plates were examined each day for 3 days. The presence of growth was quantitated (no growth or 1+, 2+, 3+, or 4+ growth), and measurements for colony size were recorded. After 1 day of incubation, well-isolated colonies were measured with a micrometer which was placed in the eyepiece of a dissecting microscope. Five isolated colonies from each plate were measured, and the average colony size was recorded in millimeters.

RESULTS

Of 117 anaerobes isolated from 42 positive clinical specimens, the major groups recovered were the *B. fragilis* group (n = 50; 43%), *Bacteroides* spp. (*B. melaninogenicus* group, *B. disiens*, and *B. bivius*; (n = 18; 15%), *Fusobacterium* spp. (n = 14; 12%), *Peptostreptococcus* spp. (n = 10; 8.5%), *Clostridium* spp. (n = 18; 11%), non-spore-forming gram-positive rods (n = 5; 4%), and anaerobic gram-negative cocci (n = 2; 2%).

The data in Table 1 compare the ability of each of the test brucella media to recover anaerobic isolates from clinical specimens at 1, 2, and 3 days. On day 1, Anaerobe Systems PRAS brucella plates recovered 61% of the total clinical anaerobic isolates, whereas the BBL, Remel, and Scott media recovered 32, 34, and 49%, respectively. *P. magnus*, *P. anaerobius*, *P. asaccharolyticus*, *F. necrophorum*, *F. nucleatum*, *B. melaninogenicus*, and *E. limosum* failed to grow or grew poorly after 1 day on Remel, BBL, and Scott brucella plates. Small colony sizes and quantitatively fewer organisms were noted on media from BBL, Remel, and Scott

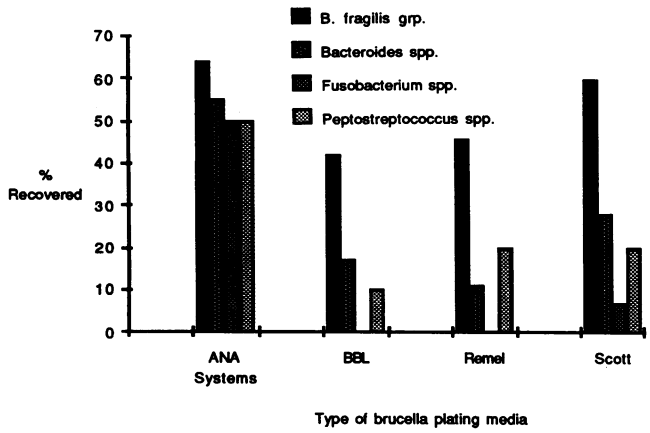

FIG. 1. Percentages of *B. fragilis* group, *Bacteroides* sp., *Fusobacterium* sp., and *Peptostreptococcus* sp. isolates recovered on four different brucella plating media after 1 day of incubation.

(1+ to 2+ growth) compared with Anaerobe Systems plates (2+ to 3+ growth). *Clostridium* spp. and the *B. fragilis* group grew well on all of the media, but they produced noticeably larger colonies on Anaerobe Systems PRAS plates after 24 h. On day 2, many anaerobes still failed to grow on BBL and Remel plates. Growth (1+) of *F. nucleatum*, *F. necrophorum*, *P. anaerobius*, and *E. limosum* appeared by day 2 on Scott plates.

By day 3, Anaerobe Systems PRAS plates recovered 94%, Scott plates recovered 75%, Remel plates recovered 61%, and BBL plates recovered 56% of the anaerobes. *P. magnus* failed to grow on BBL and Remel plates, and *E. limosum* failed to grow on Remel plates. *B. vulgatus* was missed three times on the Anaerobe Systems PRAS plates but were recovered on Scott plates, and one isolate each of *Veillonella parvula* and *C. perfringens* was recovered on Remel and Scott plates but not on Anaerobe Systems PRAS plates.

The percentages of the major clinical anaerobic isolates detected on the four plates after 1 day are shown in Figure 1. Anaerobe Systems PRAS plates recovered most of the *B. fragilis* group, *Bacteroides* spp., *Fusobacterium* spp., and *Peptostreptococcus* spp. in 24 h. Several groups (*Bacteroides* spp., *Fusobacterium* spp., and *Peptostreptococcus* spp.) showed considerable quantitative differences in recovery from the four brucella media in 1 day. Most significant was the recovery of *Fusobacterium* spp. from the brucella media; the Anaerobe Systems medium recovered 50%, the Scott medium recovered 7%, and both the Remel and BBL media recovered 0% after 1 day of incubation.

Table 2 summarizes the average colony sizes in millimeters of the ATCC anaerobic stock cultures after 1 day of incubation on the different brucella media. Anaerobe Systems PRAS plates supported good growth of all of the stock cultures (2+ to 3+), and organisms which grew on all of the plates had colonies which were 25 to 30% larger on PRAS plates. BBL plates failed to support *F. mortiferum*, *F. nucleatum*, *P. anaerobius*, *P. asaccharolyticus*, and *P. magnus* growth; Remel plates did not support *F. mortiferum*, *F. necrophorum*, *F. nucleatum*, and *P. anaerobius* growth; and Scott plates did not support *F. necrophorum* and *P. anaerobius* growth after 1 day of incubation.

DISCUSSION

Anaerobic infections are often associated with severe morbidity and may require prolonged hospitalizations. Rapid isolation and identification of anaerobes from clinical specimens can provide information earlier regarding the correct course of clinical treatment and thus reduce morbidity and length of stay in the hospital (4). It has been shown by Bourgault et al. (1) that early antimicrobial susceptibility test results enable physicians to initiate appropriate therapy earlier in a patient's illness. The alternative, delayed or ineffective treatment, may lead to prolonged and costly hospitalizations, an important consideration in this era of prospective payments and diagnosis-related groups. The selection of appropriate media for primary isolation and propagation of anaerobes from clinical specimens is an important decision that must be made by microbiologists. A medium that enhances the growth of anaerobes and produces a larger colony size permits microbiologists to provide earlier reports on the presence, identity, and susceptibility test results of anaerobic isolates to assist in the management of infection.

The present study showed that Anaerobe Systems brucella PRAS plates were superior to the other test media evaluated. Colonies appeared earlier, quantitatively there were more colonies per plate, and the colony sizes were larger and easier to detect. The superiority of Anaerobe Systems brucella medium is attributed to the manufacturing method (PRAS) and to packaging of the plates in aluminum foil pouches under strict anaerobic conditions. The alumi-

TABLE 2. Colony size of 12 ATCC stock cultures on four brucella media after 1 day of incubation

Organism	Diam (mm)[a] in medium from:			
	Anaerobe Systems	BBL	Remel	Scott
Bacteroides fragilis ATCC 25285	1.0	0.3	0.3	0.6
B. melaninogenicus ATCC 15930	0.5	0.3	0.3	0.3
B. ovatus ATCC 8483	1.1	0.5	0.4	0.5
B. thetaiotaomicron ATCC 29741	1.1	0.5	0.5	0.7
Clostridium difficile ATCC 9689	1.1	0.6	0.5	0.8
Fusobacterium mortiferum ATCC 25557	0.7	NG	NG	0.2
F. necrophorum ATCC 25286	0.6	NG	NG	NG
F. nucleatum ATCC 25586	0.5	NG	NG	0.2
Peptostreptococcus anaerobius ATCC 27337	0.7	NG	NG	NG
P. asaccharolyticus ATCC 14963	0.6	NG	0.2	0.2
P. magnus ATCC 29328	0.6	NG	0.2	0.3
Propionibacterium acnes ATCC 11827	0.4	0.2	0.2	0.3

[a] Average of five strains after 1 day of incubation. NG, No growth.

num foil pouches eliminate deterioration of the medium because of exposure to oxygen until the time of use.

Earlier studies by Murray (10) with media manufactured and stored aerobically demonstrated no significant difference in the quantitative growth of 20 anaerobes inoculated onto media that were either reduced before inoculation or contained reducing agents incorporated in them. Also, no difference was noted in the growth rates of anaerobic organisms on media either stored at 4°C for 4 weeks or freshly prepared. However, Murray did not investigate media which were manufactured and stored prereduced. Other studies have reported a distinct advantage to the use of either freshly prepared media (6) or PRAS media (9). Sondag et al. (13), who evaluated the recovery of clinical anaerobic isolates on selective and nonselective agar media, found that only 19% of the anaerobes were detected after 1 day and 70% were detected after 2 days. In addition, only 19% of *B. fragilis* group isolates were recovered after 1 day and 60% of *B. fragilis* isolates were recovered after 3 days of incubation. In the present study, 64% of the *B. fragilis* group, 55% of the *Bacteroides* spp., 50% of the *Fusobacterium* spp., and 50% of the peptostreptococci were detected after 1 day by the prereduced Brucella agar manufactured by Anaerobe Systems. As in the study by Murray (10), Sondag et al. (13) did not investigate PRAS plating media.

Previous studies by Carlsson et al. (2) and Frolander and Carlsson (5) showed the bactericidal effect of bacteriological media exposed to atmospheric oxygen. Toxic hydrogen peroxide formation and generation of superoxide and/or hydroxyl radicals are discussed. Exposure of bacteriological media containing reducing agents to oxygen also causes formation of organic peroxides that inhibit the growth of many anaerobes (12). These studies suggest that exposing media or reducing agents in media to air inhibits the recovery, total yield, and colony size of anaerobes. When oxidation of culture media occurs, any attempt to reduce the media, such as placing the medium in an anaerobic jar or chamber, does not eliminate oxidized products or cause any benefit to the medium (7, 12). Saturation of the agar medium with oxygen (as with plates manufactured and stored aerobically) may contribute to a long lag phase and the small colony size seen among the media tested here.

In conclusion, the results of these studies suggest that since the same basal medium and formulation were used in each of the media evaluated, the method of manufacture and packaging of the Anaerobe Systems PRAS brucella plates is responsible for the increased recovery and faster growth of anaerobes. Many of the anaerobes, particularly the *B. melaninogenicus* group, *Fusobacterium* spp., and peptostreptococci, are sensitive to oxidized products and thus grew better on the Anaerobe Systems PRAS brucella plates. The results of this study show that use of Anaerobe Systems PRAS brucella medium in clinical laboratories will increase the recovery and produce faster growth of anaerobes.

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